C-TRIUMPH: COHORT FOR TB RESEARCH BY THE INDO-US MEDICAL PARTNERSHIP MULTICENTRIC PROSPECTIVE OBSERVATIONAL STUDY

FUNDING: INDO-U.S. VACCINE ACTION PROGRAM

(VAP) INITIATIVE ON TUBERCULOSIS (TB) RESEARCH

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CONTENTS

PROTOCOL SUMMARY

TITLE	:	C-TRIUMPh: Cohort for TB Research by the Indo-US Medical Partnership
DESIGN	:	Multi-centric Prospective Observational Study
POPULATION	:	(i) Active TB Cohort (Cohort A): 1200 adults and children with active TB (800 adults with newly diagnosed PTB starting Category I Rx (85% being AFB smear or Gene Xpert positive) + 200 adults with EPTB +200 children with TB) (ii) Household (HH) Contact Cohort (Cohort B): 3200 adult and children -house hold contacts of the adults with active PTB (iii) Control Cohort (Cohort C): 300 adults and 200 children – without a h/o of known TB contact100 households. Each site will enroll approximately 50% of the sample
STUDY SITES	:	(i) National Institute for Research in Tuberculosis (NIRT), Chennai (ii) Byramjee Jeejeebhoy Medical College CTU (BJMC), Pune
FOLLOW UP DURATION	:	(i) Active TB Cohort: Each participant will be followed up for 24 months (ii) Household Contacts cohort: Each participant will be followed up for 24 months (iii) Control Cohort: Each participant will be followed up for 12 months
OBJECTIVES	:	C-TRIUMPh will undertake both epidemiologic/clinical and basic laboratory research studies that will address *three* specific aims - Aim 1: To measure the host and microbial factors associated with TB treatment outcomes in Indian adults and children (Active TB cohort) Aim 2: To investigate the host and microbial factors associated with progression from infection to active TB disease in adults and children. (Household Contacts) Aim 3: To explore the host and microbial factors associated with TB transmission. (Household Contacts and Control Cohorts)

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. INTRODUCTION

India has the world's highest burden of TB. An estimated 2 billion are MTB infected, 9 million develop active TB disease each year, resulting in 1.7 million deaths. Left untreated, each person with active TB will infect on average 10-15 people every year. India, with 40% of its 1.2 billion populations latently infected with MTB and 2.3 million developing active TB disease each year, accounts for 26% of the world's entire burden of TB. In addition, an estimated 99,000 active TB cases are drug-resistant TB (MDR and XDR TB) and recently India became the third country to report totally drug resistant TB (TDR) [1]. TB places a huge economic burden in India with direct and indirect costs estimated at $23.7 billion annually.

2.2. FACTORS ASSOCIATED WITH POOR TB TREATMENT OUTCOMES

Relative impact of potentially modifiable host factors (e.g. HIV, DM, smoking, IAP, alcohol misuse, under-nutrition, mental health status) on treatment outcomes such as failure, recurrence or death is not well established in populations including Indians. While most patients treated for TB are cured of TB, poor outcomes such as treatment failure, recurrent disease and death remain important challenges. Approximately 85% of Indian patients who are treated for active smear-positive PTB via the Directly Observed Therapy Strategy (DOTS) are cured (AFB smear and culture negative). However, 2% fail (remain smear positive at 5 months of treatment), 6%die on treatment, and the remainder default or transfer out. Furthermore, approximately 12% of cured patients develop recurrent TB within 18 months of treatment, either due to relapse of the same strain or re-infection from others in the community. A patient's second course of TB treatment is more than twice as likely to fail, and more than 4 times as likely to involve MDR-TB. As shown in *Table 1*, poor treatment outcomes are linked to potentially modifiable host factors though with variable quality of evidence and estimations of individual contributions.

HIV has clearly been shown to increase TB treatment failure, death and recurrence and low CD4 counts increase that risk further [2,3]. While HIV is an important risk factor at the individual level, it is not likely to be a primary driver of poor treatment outcomes in India's TB epidemic as only 0.3% of India's population has HIV and only 2-15% of Indian patients with TB have HIV co-infection.

Though **Diabetes Mellitus (DM)** increases risk of TB two-fold compared to 22 times for HIV, DM is estimated to account for at least 20% of smear positive PTB cases in India [4-6], making it a more relevant risk factor in this setting. Most but not all data suggest that DM is associated with reduced 2-3 month sputum conversion, increased mortality (pooled RR of 1.89 based on 23 studies) [7] and may be a risk factor for TB recurrence and relapse (RR of 3.89) [7] but few studies have adjusted appropriately for confounders. It is unknown if DM is associated with acquired drug resistance.

In India 44% currently use **tobacco** (11% smoke, 33% use smokeless tobacco), and 70% of rural Indians and 20% of urban Indians are exposed to indoor air pollution (IAP), which results from burning of biomass fuels such as wood and cow dung) [8,9]. Despite heterogeneity in population based data, meta-analyses indicate that tobacco smoking is associated with TB disease severity and TB-related mortality; one Indian study demonstrated 50% of TB deaths were due to smoking.[10,11] Tobacco smoke alters T cell responses, modulates mycobacterial containment, and impairs macrophage cytokine responses [12,13] and nicotine itself has systemic immunomodulatory impact and therefore smokeless tobacco may be associated with adverse TB treatment outcomes. IAP exposure generates particulate matter, carbon monoxide, and numerous other potentially toxic compounds like polycyclic aromatic hydrocarbons (PAHs) [14].Furthermore, IAP like tobacco smoke, interferes with mucociliary clearance, decreases several antibacterial properties of lung macrophages such as adherence and phagocytic rate, and has been associated with poor lung health (e.g. COPD/ bronchitis, asthma exacerbations)[14]. While there is a biological plausibility to suggest a link between tobacco, IAP exposure and TB, few studies to date have adequately measured these exposures and prospectively assessed their relationship to TB treatment outcomes, while accounting for important confounders like alcohol.

Many other factors may affect TB treatment outcome. In India, rates of alcohol use is on the rise, and 50% of adults and children are undernourished (low BMI or low weight-for-age), 30% are estimated to be vitamin D deficient, 6% depressed, and 20% food insecure [15, 16].**Alcohol**, particularly heavy alcohol use, directly and indirectly impairs the cell-mediated immunity and macrophage functions which are essential for host response to TB [17]. Alcohol use also impairs **TB treatment adherence** and causes micro- (i.e. Vitamin deficiencies) and macronutrient malnutrition (e.g. **low BMI**), which independent of alcohol use, have been negatively associated with TB immunity [18]. Moreover, deficiency of Vitamin D may cause reduced sputum smear conversion and increased inflammation during TB treatment [19]. Low BMI has been associated with treatment failure, death and recurrence [17, 20]. It is unknown what role other nutritional deficiencies or household (HH) **food insecurity** play in TB treatment outcomes. **Mental Health** status, particularly depression, may also influence treatment adherence and reduce immunity [17, 21, 22].Therefore, careful ascertainment of alcohol, treatment adherence, nutrition status, HH food insecurity and depression is critical.

While non-adherence and **suboptimal plasma concentrations of anti-TB medications** are also likely contribute to poor TB treatment outcomes, target concentrations of first-line TB drugs are based on relatively limited evidence [23-26]. Importantly, treatment outcomes differ by geographical setting, complicating evaluation of relationships between concentration-effect relationships for TB drugs [27]. In India, for example, slow INH metabolizer genotype is common, so, theoretically, low INH concentrations may be relatively uncommon; however, malnutrition and DM are common and can lead to low rifampicin concentrations [28]. Furthermore, there are few studies evaluating the evolution of drug resistance among individual patients and the relationship between drug exposure and acquired resistance [29]. Many **socio-behavioral factors** influence drug adherence too - stigma, mental health, literacy, social support, substance abuse etc. – and, thereby, influence drug exposures.

Non-modifiable host factors like age, sex, human genetic polymorphisms, novel proteins and microbial factors (e.g., mycobacterial strain types) are also likely to influence TB treatment outcomes. Human genetic variants (such as that of leukotriene A4 hydrolase-*LTA4H*) [30, 31] may impact treatment outcomes. Animal model studies have also identified peptidoglycan recognition proteins (PGYRP1-4) [32-34] and nucleotide binding oligomerization domain-containing proteins (NOD1, NOD2) [35, 36] are directly bactericidal against many gram positive and gram negative bacteria. NOD2 has been shown to be involved with detection and defense response to MTB but the role of the others in TB is unknown. Thus the prevalence of *LTA4H* or expression of PGYRP or NOD proteins may differ between persons who have non-relapsing cure versus those who do not but this needs further investigation. There is also mounting evidence that MTB has more sequence variation than previously estimated and that some of this genetic diversity may actually have important clinical phenotypic sequalae [37].

Animal studies have shown that MTB strains can differ in virulence and immunogenicity [38]. For example guinea pig studies from the early 20thcentury observed that MTB strains from Indian TB patients were less virulent than strains from United Kingdom patients [37]. Beijing genotype MTB strains are linked to increased likelihood of AFB sputum smear positivity [37]. Therefore, strain types may influence treatment outcomes, the ability to cause disease and to transmit within certain geographic settings or ethnic groups [39].

2.3. FACTORS ASSOCIATED WITH PROGRESSION TO ACTIVE TB DISEASE

Many of the above-mentioned potentially modifiable factors have been associated with progression to active TB, but their relative independent and combined contributions towards risk of active TB disease has not been well studied, particularly in India. HIV increases the risk of reactivation of latent MTB infection by up to100 times and increases the risk of progression to active disease in those who acquire new MTB infection [40]. Other less potent but more common risk factors including DM, under nutrition, smoking and alcohol misuse have been associated with 2-3 times increased risk of development of active TB [41- 43].

Non-modifiable host factors such as age, sex, and host genetics may also be influential. Disease risk after primary infection is highest in children < 4 years, then decreases until age 10, after which a second peak starts and is noted through reproductive age until 34 years in women but is more sustained in men until age of 50 years [44]. These age- and sex-specific differences also impact the presentation of disease where young children are more likely to have disseminated disease while women are more likely than men to have EPTB. The mechanisms for these differences are unknown. Host genetic polymorphisms and host proteins may also be associated with risk of developing active TB, [45, 46] but it is unknown what the prevalence or association is of LTR4 genetic variants or PGYRP or NOD proteins with risk for developing active TB. Microbial strain lineage may also matter. A study among Gambian TB patients and their household contacts found that progression to active TB but not transmission varied by MTB lineage with *M.africanum* causing lower proportion of active TB disease than other MT strains [38]. Further,

strain lineage has differing phylogeography, where India, sub-Saharan Africa and the Americas each have different predominant strain lineages [37]

2.4. FACTORS ASSOCIATED WITH TB ACQUISITION AND TRANSMISSION

There is a variable response to exposure to infection. Many of the same host and microbial factors described above are associated with transmission of infection, but may have different relative contributions (**Table 1**). In addition, proximity to the index case, characteristics of the HH and duration of exposure are important. Host genetics may also play a role. Further, MTB transmission dynamics appear to vary by MTB strain type as demonstrated by Albanna et al [47] who found that east African Indian MTB strains were associated with reduced transmissibility compared to other MT lineages.

2.5. MULTIPLE HOST AND MICROBIAL FACTORS ARE LINKED TO TB TREATMENT OUTCOMES, PROGRESSION AND TRANSMISSION

While the quality and strength of the evidence varies substantially, implicated *host factors* include social (e.g. residence in crowded urban setting, low socioeconomic status), environmental (e.g. IAP), and clinical/biological (e.g. age, sex, co-morbidities such as HIV, DM, tobacco, alcohol, malnutrition, depression, genetics) determinants. *Microbial factors* include strain type (e.g. Beijing vs. other), drug-resistance pattern, as well as genes and proteins associated with virulence, persistence, and evasion of host immune responses. Treatment outcomes are also impacted by drug adherence and serum concentrations of TB drugs, which in turn are influenced by many of the above-mentioned host related factors.

2.6. NEED FOR C-TRIUMPH STUDY

Studies to date have not included a prospective comprehensive assessment of these multiple host and microbial factors to accurately examine their independent and combined attributable impact on outcomes of TB treatment, progression and recurrence, and transmission. Since short-term microbiologic outcomes (like 2-month sputum culture conversion to negative) correlate poorly with long-term treatment outcomes (cure without relapse), a prospective cohort that follows patients for at least 18 months post-treatment is recommended to assess clinically-relevant treatment effects.

Understanding the relative importance of potentially modifiable and non-modifiable factors will help to inform which of them should be prioritized for public health interventions to reduce the burden of TB in India and other settings. We will also be able to provide much needed data on interaction and combined risk of these factors which can be used for improved TB epidemic modeling. Establishing a specimen repository for host and microbial studies, archiving MTB strains from prevalent and incident TB cases will allow for molecular epidemiological typing and further exploration of associations between MTB strain type and clinical phenotypes such as progression to active TB. These MTB strain libraries are also valuable for future selection of novel targets for mycobacterial diagnostic tests and TB vaccine development

3. Objectives

The study will establish and maintain 3 prospective cohorts -- adults and children with active TB disease **(1.Active TB Cohort)**, adults and children at high risk for developing active TB **(2.Household Contacts Cohort**), and adult and children controls who do not have any known exposure to TB **(3. Control Cohort**) to address our 3 specific aims-

Aim 1: To measure the host and microbial factors associated with treatment outcomes in Indian adults and children

Aim 2: To investigate the host & microbial factors associated with progression from infection to active TB disease in Indian adults and children

Aim 3: To explore the host and microbial factors associated with MTB transmission

TABLE 1. C-TRIUMPH – STUDY OBJECTIVES AND TIMEFRAME

	KEY OVERARCHING SCIENTIFIC QUESTIONS			
	HOST FACTORS Which host factors, individually or combined, are associated with TB treatment outcomes, disease progression and transmission in India?		**MICROBIAL FACTORS** Which microbial factors, individually or combined, are associated with TB treatment outcomes, disease progression and transmission in India?	
	SCIENTIFIC HYPOTHESES			
	H_0 *Potentially modifiable (HIV, DM, tobacco, IAP, alcohol, undernutrition) and non-modifiable host factors (age, sex, genetics) alone or in combination have the greatest association with TB treatment outcome, disease progression and transmission in India*		***H_0*** *Specific Mtb strain lineages and drug susceptibility patterns are most highly associated with TB treatment outcome, disease progression and transmission in India*	
	PRIORITY RESEARCH STUDIES			
	Clinical/Epi Studies	**Lab Studies**	**Clinical/Epi Studies**	**Lab Studies**
Specific Aim 1 Treatment Outcome	1. Prevalence and relative contributions of HIV,DM, tobacco, IAP and other modifiable risk factors on TB treatment outcome *(Year 2-4)* 2. Population PK of first line TB drugs, factors associated with their concentrations (NAT2, SLCO1B1 genotype, BMI, DM, HIV, etc), and treatment outcome *(Year 3,4)* 3. Hair samples to measure PK of first line TB drugs and correlate with drug adherence and plasma drug concentrations *(Year 2-4)*	1. Functional genetic variants in Indian populations within LTA4H and other components of eicosanoid and TNF pathways and associations with treatment outcomes *(Years 4,5)* 2. Whole blood transcriptome, proteosome, clinical presentation *(Year 3)* and treatment outcome *(Year4)* 3. Differential expression of PGLYR and NOD proteins in active TB *(Year 3)*	1 Mtb strain lineage, drug susceptibility, clinical presentation *(Year 3)* and treatment outcome *(Year 5)*	1. Transcriptome and lipid profile of serial Mtb strains from patients on treatment *(Years 3,4)* 2. Whole genome sequencing to assess microbial genes associated with PTB vs CNS TB in adults and children *(Years 4,5)*
Specific Aim 2 Disease Progression	1. Host markers of inflammation, oxidative stress, IAP, nicotine and risk of development of active TB *(Year 3)* 2. Impact of HIV, DM, tobacco, IAP and other modifiable risk factors on development of active TB *(Year 5)* 3. Estimate risk of disease progression by TST/QGIT status in adults and children *(Year4,5)*	1. Biosignatures of latency and active TB *(Year3,4)* 2. Host response to 16 novel Mtb antigens for diagnosis of LTBI and active TB *(Year 3,4)* 3. Differential expression of PGLYR and NOD proteins in different stages of infection *(Year 3,4)*	1. Compare index Mtb and HH incident TB strains to determine if specific Mtb strain types are associated with development of active TB *(Years 4,5)*	1. Microbial transciptomebiosignatures associated with progression to active disease *(Years 3,4)*
Specific Aim 3 Transmission	1. Within HH transmission dynamics *(Year 4,5)* 2. Relative contribution of HIV, DM, smoking, IAP and other modifiable risk factors on TB transmission*(Year 5)*	1. Host sputum and peripheral blood inflammation markers, sputum macrophage function, in IAP and/or tobacco exposed and risk of increased host susceptibility *(Years 3,4)*	1. Compare Mtb strain sequences and drug susceptibility patterns of transmitters vsnontransmitters*(Years 4,5)*	1. Microbial transciptomebiosignatures associated with transmission of Mtb infection *(Years 3,4)*

4. STUDY DESIGN

4.1. STUDY TYPE

Multi-centric Prospective Observational Study

4.2. STUDY DURATION

5 years

4.3. STUDY POPULATION

3 prospective cohorts - Active TB, HH Contacts and Controls will be established

i. **ACTIVE TB COHORT** will include adults with newly diagnosed active pulmonary TB (PTB) starting RNTCP Category I treatment (with at least 85% being AFB smear or GeneXpert positive at entry),adults with extrapulmonary TB (EPTB), and children with TB.

ii. A **HH contact** will include all adults and children living in the same house as the adult (>18 yrs) active PTB participant during the 3 months prior to diagnosis of TB

iii. **CONTROL HHS** will be randomly selected from within a 5km radius of the Index case HH.

4.4. STUDY SITES

NIRT Chennai –The NIRT clinical site will be enrolling cohort patients attending RNTCP clinics at the primary health centers (PHCs) of 2 selected Tuberculosis units (TU) in Tiruvallur district. There are 6 TB units (TU) in Tiruvallur district. For the proposed Active TB cohort, we will enroll TB patients attending 2 of these TU's namely Velliyur and Poonamalle TU's.

BJMC Pune – The BJMC site will be enrolling cohort patients attending RNTCP clinics at BJ Medical College and the referrals from the other DOT centre's in the city. There are two potential sources for identifying new active TB cases at BJMC. These include the BJMC RNTCP outpatient clinic (OPD), which receives referrals from the community as well as from other OPD's at BJMC where patients with TB present, including the BJMC NACO HIV ART Centre (the largest HIV treatment centre in Pune); the antenatal clinic and paediatric clinics. TB patients will also be identified following admission to the inpatient wards (adult, antenatal and paediatric).

5. STUDY POPULATION

5.1. RATIONALE FOR SELECTION OF STUDY SUBJECTS

Each of the 3 Cohorts will establish a clinical database, including socio-behavioral assessments, as well as a strategic specimen repository of samples obtained from baseline and selected follow-up time points. MTB isolates from confirmed TB cases will also be stored.

i. Active TB cohort will be used to address Aim 1 (To examine the host and microbial factors associated with TB treatment outcomes in Indian adults and children) and Aim 3 (To explore the host and microbial factors associated with MTB transmission).
ii. The HH Contact Cohort will be used to address Aim 2 (to investigate the host and microbial factors associated with the progression from infection to active TB disease in Indian adults and children) and Aim 3.
iii. The Control Cohort will be used in conjunction with the Active TB and HH Contact Cohorts to undertake exploratory studies focused on biomarker discovery for predicting progression to active disease and transmission by creating a comparison group that is similar to the active and HH contact cohorts in terms of geography and its attendant co-factors (e.g., SES).

5.2. SUBJECT INCLUSION CRITERIA

ACTIVE TB COHORT (ALL MUST APPLY)

i. Adults and children(<14 years of age)with newly diagnosed PTB; or EPTB presenting to Velliyur and Poonamalle TUs, in Tiruvallur and the BJMC RNTCP that meet RNTCP Cat-1 treatment criteria;
ii. Ability of participant/legal guardian to provide informed consent/assent;
iii. Allow home visits;
iv. Live within a 50km radius of either of the 2TU'sor the BJMC RNTCP and plan to remain in the area for the duration of the study.

Note: Consenting HH contacts from HH contact cohort with newly diagnosed PTB or EPTB who meet the inclusion criteria for Active TB Cohort may be eligible for enrollment into the Cohort A.

PTB will be either smear or Xpert-positive at entry; or smear negative at entry but culture confirmed by 12 weeks.

Note: Adult patients with PTB who are enrolled but found later to have a negative sputum culture for MTB will be followed up in the study. Feasibility of follow-up will be reviewed if the proportion of smear +ve but culture–ve cases account for 5% of total sample size (or approximately 20 participants per site).

HOUSEHOLD CONTACTS COHORT (ALL MUST APPLY)

i. All adults and children living in the same house as the adult (>18yrs) active Pulmonary TB participant during the 3 months prior to diagnosis of TB.
ii. Ability of participant/legal guardian to provide informed consent/assent

CONTROL COHORT (ALL MUST APPLY)

i. Individuals with no know prior exposure to MTB
ii. Living within a 5km radius of HH contacts
iii. Ability of participant/legal guardian to perform informed consent/assent

5.3. SUBJECT EXCLUSION CRITERIA

ACTIVE TB COHORT

i. Critical illness where life expectancy is < 1 month
ii. Children weighing less than 5kgs
iii. Presence of a medical condition that, in the investigator's opinion, would interfere with the individual's participation or interpretation of results
iv. Received > 1 week (five daily or 3 intermittent doses) of multi-drug TB therapy in the preceding 30 days
v. Received more than seven days of fluoroquinolone therapy or other drugs with anti-TB activity (e.g., rifampicin, ethambutol, clofazamine, aminoglycosides) for any reason in the preceding 30 days
vi. Failure to provide consent for enrolling HH contacts

HH CONTACTS COHORT

i. Presence of a medical condition that, in the investigator's opinion, would interfere with the individual's participation or interpretation of results
ii. Children weighing less than 5kgs
iii. Clinical signs or symptoms of active TB (persistent cough, draining lymph node or other evidence of extra-pulmonary TB, fever, weight loss or failure to thrive (children), fatigue or lethargy, night sweats, pleuritic chest pain, or hemoptysis). If clinical signs or symptoms of TB are present but CXR, sputum culture and other evaluation rules-out TB, the participant may be enrolled
iv. Not staying in the area for the duration of the study/plans to move away

CONTROL COHORT

i. Presence of a medical condition that, in the investigator's opinion, would interfere with the individual's participation or interpretation of results
ii. Children weighing less than 5kgs
iii. Not staying in the area for the duration of the study/plans to move away

6. STUDY PROCEDURES

6.1. SCREENING

Recruitment of Active TB Cohort

A study counselor or nurse will determine if each patient identified at the two study sites, meets the inclusion criteria for the Active TB Cohort study. Patients who fulfill the inclusion criteria will be invited to participate in the Active TB Cohort. Details of the study, including study objectives and outcomes, will be explained to the patients in their native language and they will be asked to provide informed written consent prior to initiation of screening procedures. Informed consent of legal guardians and assent of the child will be obtained for participants under the age of 18 years. All participants will also be asked to provide home visit consent and agree to reveal their TB status to family members for the potential recruitment of participants for the Household Contacts Cohort. The timing of all follow up visits for the study participant from Cohort A is timed from the initiation of their TB treatment

Recruitment of Household Contact Cohort

An outreach team (eg- clinician, counselor and field investigator) will approach all participants enrolled in the Active TB Cohort for the potential enrollment of family members in the Household Contact Cohort. A household contact investigation among its members will be conducted only if the index case of the household had consented to the home visit and enrolment of their family members into the study. Enrollment of a household contact will occur within two weeks to 2months of the index case from Cohort A. Individual household members will be consented for the study procedures at the initial visit at their homes. The RNTCP nurse, PHC staff or the DOTS provider who supervises the TB treatment of the index case will support and participate in community education and awareness of the study and in recruitment of household contacts of the index case. A trained outreach team comprised of a study physician, nurse and counselor will go to the home to consent patients.

Recruitment in Control Cohort

Control HH's will be randomly selected from within 5 km of the same communities as HH Contact Cohort using computer software such as the E-Pop. Assistance of Non-governmental organizations (NGO) working for TB will be sought for the enrollment of control HH's by establishing a networking system. A trained outreach team comprised of a study physician, nurse and counselor will go to the home to consent patients. For those patients who consent to participate in the study and specimen storage; baseline demographic and clinical data and specimens will be collected in the mobile van or in the clinic. Sample size =100 Households

6.2. CLINICAL ASSESSMENTS

For all 3 cohorts

Medical and Medication History: All prior TB, HIV related events, diseases of the liver, lung (e.g. asthma/COPD, pneumonia), cardiovascular system, DM, and other immunosuppressive conditions will be recorded. Medication allergies and medication history including all prescription and nonprescription medications (e.g. any Ayurvedic, Unani, Siddha or homeopathy therapies) taken within 30 days prior to study entry will be obtained. Any prior medications for TB, HIV, DM will also be recorded. Children's co-morbidities (e.g. asthma, respiratory infections, HIV), and medications for chronic conditions will be collected.

Socio-Demographic Form: The detailed socio-demographic information about the Index and all the members of the household will be captured in this form. It includes information about the marital status, education, religion, employment status as well as the health seeking behavior of the Index cases as well as the individuals in the HH and Control Cohort. In addition, information about the socio-economic details about the family and TB source evaluation is also collected from the Index case. This form will be filled at the time of enrollment from the index as well as all the members of the household and the Control Cohort.

Psychosocial Interview: Interviews of all adult index cases and the adults in HH cohort will include alcohol use (AUDIT) [48], tobacco use behaviors (Fagerstrom) [49], illicit substance use, depression scale (CESD) [51]. For children a briefer interview will be conducted. Follow-up interviews will be conducted according to the evaluation schedule for each of the three cohorts.

TB and Lung Health Assessment: Signs/symptoms of TB at entry and at follow-up including cough, fever, night sweats, involuntary weight loss, and lymphadenopathy, and other lung health signs/symptoms (e.g., phlegm, wheezing, breathlessness) will be assessed using standardized questionnaires such as BOLD (or SGRQ) [52]. Follow-up Lung Health assessments will be conducted at every study visit.

Concomitant Medications: All medications including TB preventive treatment will be recorded at entry. Following entry visit, systemic medications started or stopped since the last study visit will be recorded on the case report form (CRF), including actual or estimated start dates and stop dates.

IAP: An assessment tool developed by IAP investigators at JHU will be used for IAP evaluations. Follow-up IAP evaluations will be conducted in the at the scheduled study visits for each cohort.

Nutrition Assessment: The HIFAS HH food insecurity questionnaire will be used for the assessment of nutritional status [54]. Follow-up assessments will be performed at the scheduled study visits.

Targeted Physical Exam and Anthropometrics (post-entry evaluations): This will include vital signs (temperature, blood pressure), height/length at baseline and weight. Exam will be driven by any previously identified or new signs/ symptoms experienced since the last visit.

Assessments may happen at the home, mobile van, at the study clinic or some combination of the above based on the HH contact and Control HH preferences. All assessments for the Active TB cohort will take place at the study clinic.

For Active TB Cohorts alone

The *relative impact of host factors* including age, sex, HIV, DM, tobacco exposure, IAP exposure, alcohol, BMI, mental health, presence of cavitation, and symptom duration on treatment failure will be assessed. EPTB adult cases and pediatric TB cases will be included for secondary analyses.

A *nested pharmacology study* to evaluate the relationship between exposures of first-line TB drugs and poor TB treatment outcomes of failure and recurrence will be conducted.

Complete Physical Exam and Anthropometrics: At entry this will include an examination of the vital signs (temperature, pulse, and blood pressure), height/length, weight, skin, head, mouth, neck;, cardiac exam, abdominal exam, and lower extremities for edema.

TB medication: Type of TB regimen and self-report interviews on adherence to TB drugs using ACTG adherence questionnaire will be performed during treatment phase. Toxicity and response to treatment, adherence, time-to-AFB smear negative and culture negative status will be performed at consecutive follow-up visits.

Diagnoses/Adverse events: All diagnoses and adverse events (such as skin rash, numbness, tingling in extremities) identified by the treating clinician and/or visiting health worker will be recorded at follow-up visits.

All assessments for the Active TB cohort will happen at the study clinic.

For HH contacts cohort

A *10-item questionnaire* recently developed in South Africa that quantifies TB exposure as a reliable surrogate measure of TB infection in children will be administered [55]. All symptomatic HH contacts will be referred immediately to the clinical facility for further evaluation. Isoniazid Preventive Therapy (IPT) will be recommended for all asymptomatic children under age 5**.**

Assessments may happen at the home, mobile van, at the study clinic or some combination of the above based on the HH contact preferences.

Visit Window Periods

For each study participant, the window period for conducting a study visit is as follows:

- Cohort A: +/- 3 days for all study visits before and including two months, and +/- 7 days for all visits afterwards
- Cohort B & C: +/- 14 days

TABLE 1: SCHEDULE OF EVALUATIONS FOR ACTIVE TB COHORT[1]

CRF | Time	Entry	2W	4W	8W	5M	6M	12M	18M	24M	End of Tx[2]	TB recurrence (failure, relapse)
Consent/Enrollment	X										
Medical History	X										
Psychosocial Interview	X			X		X	X	X		X	X
Socio-demographic Interview	X										
Clinical Assessment (i.e. TB and Lung Health Assessment, Physical examination[5], Concomitant Meds, Diagnoses/AEs)/ Medication History	X	X	X	X	X	X	X	X	X	X	X
TB Medication Adherence	X	X	X	X	X	X				X	
Nutrition Assessment[4]	X					X				X	X
Anthropometrics[6]	X	X	X	X	X	X	X	X	X	X	X
Chest Radiograph[9]	X			X		X					X
Sputum AFB and culture X 2 (DST if culture+)[13]	X	X	X	X	X	X		X		X	X
Sputum for Cepheid Gene-Xpert X 2	X										X
Extrapulmonary TB sample for AFB and culture/histopathology	X[8]										X
Hematology (CBC)	X			X							X
HIV Test (if not already known)	X										X
CD4 count if HIV +ve[10]	X										X
Hemoglobin A1c (Adults)[11]	X									X	X
LFT	X		X	X						X	X
Chemistry (Renal panel, glucose)	X									X	X
Urinalysis	X										X
Urine Pregnancy test	X			X		X					X
PFT (Adults), Spirometry (Child) (random subset)				X						X	X
Samples for Biorepository	**Entry**	**2W**	**4W**	**8W**	**5M**	**6M**	**12M**	**18M**	**24M**	**End of Tx**	**TB Recurrence**
Stored Plasma sample for PK[7]			X		X						
Stored Plasma	X		X	X		X	X		X	X	X
Whole Blood for DNA		(X)[12]									
Stored Sputum	X	X	X	X		X				X	X
Stored Urine	X		X	X		X	X		X	X	X

Stored Hair	X		X		X	X				X	X
Stored Mtb isolate	X			X	X	X					X
Whole Blood for Host mRNA (PAXgene)	X		X	X		X	X		X	X	X
Whole Blood forPBMC	X		X	X		X	X		X	X	X
Whole blood for Quantiferon supernatant	X		X	X		X	X		X	X	X

[1] Active TB participants with Microbiological Confirmation of TB (defined as smear+, Xpert+, or smear negative but cultured confirmed. Patients who are initially negative by smear and/or Xpert will be enrolled if they meet criteria for Cat-1 TB treatment. Participants who remain culture negative by 3 months will be follow-up but will not be included in the primary analysis.

[2]An additional 'End of Rx' time-point to be observed for participants with Rx duration exceeding 6 months

[3] Indoor Air Pollution monitoring will be done only on a subset of cohort B households.

[4] Household food insecurity

[5] A complete physical exam at entry. At post-entry visits, a targeted physical exam will be performed

[6]For adults and children: Height / length, mid upper arm circumference, weight at baseline. At post entry visits, weight and height / length will be measured

[7]A 2 hour post TB drug dose (participant will take their dose in clinic and will have the time noted for a blood drawn 2 hours post dose administration)

[8]Additional EPTB samples to be collected at the discretion of the onsite clinical staff. These specimens are collected as part of clinical evaluation only and will not be retained/repeated for study purposes.

[9]CXR at baseline will be done unless one was taken as a part of the clinical investigation of the participant's TB through the RNTCP. Data from an 8 week and 6 month CXR will be collected if testing was done as part of the RNTCP standard of care or if participant has pulmonary disease. Pregnant women are not required to have CXR. CXR will be done at End of Tx if it didn't overlap with 6M

[10]CD4 count will only be performed on participants who have not had a CD4 count tested in the preceding 6 months.

[11] End of Treatment assessment of HbA1c will be selectively done on those participants with abnormal HbA1c at baseline

[12] Can be collected at any time-point between 2W and 6M

[13] At enrollment, one spot specimen and one early morning specimen will be obtained from all Cohort A participants. Diagnostic tests will be run on both specimens. During subsequent visits, two sputum spot specimens will be collected, and will be pooled for testing. The remaining specimens would be stored.

TABLE 2. SCHEDULE OF EVALUATIONS FOR HH CONTACTS COHORT

Time	Entry	4M	6M	12M	18M	24M	Active TB[1]
Consent/Enrollment	H						
Medical History	H						
Brief Psychosocial Interview	H			H		H	F
Brief Socio-demographic Interview	H						
Clinical Assessment[2] (i.e. TB and Lung Health Assessment, Concomitant Meds)/Medication History	H	H	H	H	H	H	F
Urine Pregnancy test	H	(H)					F
Nutrition Assessment[3]	H	H	H	H	H	H	F
IAP	H	H	H				
Targeted Physical Exam and Anthropometrics[4]	H	H	H	H	H	H	F
Chest radiograph	F	(F)					F
Sputum AFB and culture x 2 (DST if culture +)	H	H					F
TT Skin Test	H	H[5]		H[5]		H[5]	
Quantiferon Gold in Tube	H	H[5]		H[5]		H[5]	F
CBC	H						
Rapid HIV Test[6]	H						F
Hemoglobin A1c Point of care (Adults)	H			H			F
Household Air Sample (random subset)	H	H		H			
PFT (Adults), Spirometry (Child) (random subset)	H			H			
Samples for Biorepository							
Stored Plasma	H	H		H			F
Stored DNA	H						
Stored Sputum	H	H					F
Stored Urine	H	H		H			F
Stored Hair	H	H		H			F
Stored MTB isolate	H						F
Stored QGIT supernatant	H	H		H		H	F
Stored Host mRNA (Paxgene)	H	H[7]		H[7]			F
Stored PBMC	H	H[7]		H[7]			F

F=Facilities-based activity; (F)= Facility Based activity only if indicated; H= Home-based activity; M=months

[1] Any person who has active TB (prevalent or incident) is eligible for the Active TB Cohort if he or she consents. Active TB assessment will otherwise be done as included in this SOE if the participant does not enroll in the Active Cohort.

[2] All symptomatic household contacts will be referred immediately to the clinical facility for further evaluation. IPT will be recommended for all asymptomatic children under age 6 and all asymptomatic HIV+ household contacts

[3] Household food insecurity and dietary questionnaire

[4] Height, mid upper arm circumference, weight at baseline; at post entry visits, weight will be measured

[5] TST and QGIT will only be done if prior was negative

[6] HIV testing will only be done on adults. If there is a HIV+ adult in the household, then the legal guardian of the child will be asked if they want their child to be HIV tested

[7] Host mRNA, PBMC, saliva, will be performed on all at baseline and then a random subset of 200 households (n=800 contacts)

TABLE 3 . SCHEDULE OF EVALUATIONS FOR CONTROL HOUSEHOLDS COHORT

Time	Entry	4M	12M
Consent/Enrollment	H		
Medical History	H	H	H
Brief Psychosocial Interview	H		H
Brief Socio-demographic Interview	H		
Clinical Assessment[1](i.e. TB Assessment, Concomitant Meds)/ Medication History	H	H	H
Nutrition Assessment[2]	H	H	H
Urine Pregnancy Test	H		
Targeted Physical Exam and Anthropometrics[3]	H	H	H
TT Skin Test	H	H[4]	H[4]
QGIT	H	H[4]	H[4]
CBC	H		
Rapid HIV Test[5]	H		
Hemoglobin A1c Point of Care (Adult)	H		
IAP & HH Air Sample (random subset)	H		
Samples for Biorepository			
Stored Plasma	H	H	H
Stored DNA	H		
Stored Sputum	H	H	H
Stored Urine	H	H	H
Stored Hair	H	H	H
Stored Stimulated QGIT	H	H	H
Stored Host mRNA (PAX gene)	H	H[6]	H[6]
Stored PBMC	H	H[6]	H[6]

H= Home-based activity; M=months

[1] Controls have to be without evidence of TB or acute illness to be eligible at entry. If during follow-up, any control is noted to have clinically concerning symptoms (e.g. fever, cough, diarrhea) then sample will not be collected and they will be referred for further care.

[2] Household food insecurity and dietary questionnaire

[3] Height, mid upper arm circumference, weight at baseline; at post entry visits, weight will be measured

[4] TST and QGIT will only be done if prior was negative

[5]HIV testing will only be done on adults. If there is a HIV+ adult in the household, then the legal guardian of the child will be asked if they want their child to be HIV tested

[6] These additional samples will be taken at baseline from all and a random sample of 20 households (100 people) at time points shown in green

6.3. LABORATORY EVALUATIONS

Sputum studies: Two sputum specimens (approximately 5 ml each/visit) will be collected at baseline, one spot and one early morning will be collected from adult active TB cases and HH contacts and will be processed for AFB smear and liquid media cultures (MGIT). On active TB cases only, Cepheid Gene Xpert MTB RIF will also be done. A drug sensitivity test (DST) will be performed if culture is positive.

For participants in the Active TB Cohort, samples will be taken at baseline (BL), 2, 4, 8 weeks (W),5 months,6 months (M), end of treatment and at recurrence. For all visits after enrollment two spot/early morning specimens will be collected one for testing and one for storage.

For household contacts: BL and 4M for Processing and storage

For Control Cohorts : BL, 4M & 12M for storage

For children < 5 years who are active TB cases, two gastric aspirates will be collected and will be processed for smear, rapid TB identification by Xpert MTB RIF assay and MGIT. For children in contact and control HHs, if a TB symptom screen is positive then they will be referred to the clinic to have sputum of gastric washing studies done as age-appropriate.

EPTB samples: Additional smear and cultures and histopathology of extra pulmonary specimens will be performed as clinically indicated.

Urine samples: Up to 10 ml will be taken at each visit. For participants in the Active TB Cohort, samples will be taken at BL, 4 and 8W, 6M, end of treatment, at recurrence and an additional sample will be taken at BL for urinalysis. For household contacts and controls, samples will be taken at BL and 4 and 12 months for storage purposes. Dipstick urinalysis will be performed with microscopic examination as necessary.

Females of reproductive potential (girls who have reached menarche, women who have not been post-menopausal for at least 24 consecutive months, (i.e., who have had menses within the preceding 24 month), or women who have not undergone surgical sterilization, hysterectomy or bilateral salpingectomy or bilateral oophorectomy or tubal ligation) must receive urine pregnancy test at the time of enrollment and a card pregnancy test will be performed before chest xrays taken.

Chest radiograph: CXR will be performed at baseline unless one was taken as a part of the clinical investigation of the participant's TB through the RNTCP. Data from an 8 week and 6 month CXR will be collected if testing was done as part of the RNTCP standard of care or if participant has pulmonary disease.

Pulmonary Function Tests/ Spirometry: Diagnosis of asthma/COPD will be performed on a random subset using portable PFTs (adults) and spirometry (children >5 years) to corroborate self-report as well as measure post-treatment lung health in Active TB Cohort and risk of development of TB in Contact Cohort.

Blood samples: Blood samples will be collected for storage and safety laboratory tests. Maximum of up to 28ml of blood will be taken from a vein for adults (age group >14 years) during study visits. Up to 20ml will be stored for future tests while remaining will be used for safety tests and CD4 counts. For children, the total volume of blood being collected shall not exceed 3ml/kg body weight in an 8 week period.

For storage -

For participants in the Active TB Cohort, samples will be taken at BL, 4 and8W, 5M, 6M, 12 month, 24 months, end of treatment, and at recurrence.
For household contacts and controls, samples will be taken at BL and 4, 12 and 24 months.
For control cohort, samples will be taken at BL and 4 , 12months.

For safety labs – following tests will be performed -

a) Hematology: Complete Blood Count (CBC) with differential will be obtained for both all 3 cohort participants.
b) HIV Studies: If HIV status is unknown, HIV testing will be performed on consenting adult participants. Children less than 18 years will only be tested for HIV if they are active TB cases or if a parent has tested positive or is known to be HIV-infected.

c) Hemoglobin A1c for DM: Hemoglobin A1C, a recommended test for DM will be done for all adult participants of all three cohorts.

d) Chemistry: *Renal panel:* Blood urea nitrogen (BUN), creatinine;.
Liver Function Tests (LFTs): Total bilirubin, AST, ALT, alkaline phosphatase and glucose.

Tuberculin Skin test (TST): A TST skin test will be placed and read ideally 48-72 hours (but within 1 week is acceptable) of placement on all HH contacts and controls. It will only be performed at follow-up visits if prior results were negative.

Quantiferon Gold in Tube (QGIT):Approximately 3 mLs of blood will be collected and processed for QGIT in Active TB cohort participants at BL, , 4W, 8W, 6M, 12M, 24M, end of treatment and at recurrence; in HH cohort participants at BL, 4M, 12M, 24M, TB activation and in control cohort participants at BL, 4M and 12M.

HH Exposure to IAP: HH samples will be collected on a random subset to estimate air particle concentration (PM 2.5) and carbon monoxide as an objective marker for exposure to IAP from biomass fuels and corroborated with IAP questionnaire data.

Sample of hair: A small thatch of hair (~30-50 strands) will be collected for the hair sample.
For Active TB Cohort hair sample will be taken at BL, 4W, 5M, 6months, End of Tx and Recurrence;
For household contacts and controls: BL and 4 and 12 months.

7. PROPOSED SPECIMEN REPOSITORY

This section will describe the purpose and specific details for collecting blood, urine, and sputum specimens from participants in each of the three cohorts (active TB cohort, HH contact cohort and control cohort) for placement in biostorage for future studies. The samples will be curated, stored, and managed at the Central Repository - National Institute for Research in Tuberculosis, Chennai. All participants will be consented for long term storage of samples. The resultant "bank" of biological samples will be made available to investigators participating in the RePORT-India Consortium through a peer-review process that considers high-priority, credible proposals for their use. Example purposes for stored specimens (reference Am J RespirCrit Care Med 184: 972-979, 2011) include but are not limited to:

- **M. Tb isolates** for full genome sequencing for virulence factors, association with clinical outcomes, and in the case of relapse, for comparison to the baseline specimen.
- **Plasma** for proteomics, metabolomics, lipidomics, and noncellular measures of immune response (i.e., cytokines, chemokines).
- **Whole blood** for transcriptomics.
- **Whole blood stimulated with mycobacterial antigens** yielding supernatant to be stored for measuring non-cellular immune responses, e.g., chemokines, cytokines.
- **Peripheral blood mononuclear cells (PBMCs)** to measure CD4, CD8 and other cellular immune responses.
- **Urine** for metabolomics and measures of microbial markers.
- **Sputum** for mRNA, microbiologic measures and host immune markers.

While this valuable resource will be primarily for use within India, it is expected that the "bank" will also be available to investigators external to the Consortium if they propose to work together with a Consortium member or group of sites and assuming that the appropriate ethical and scientific approvals are obtained.

Since this study requires collection of additional samples and testing beyond what is normally collected or tested for participants being treated in the Revised National TB Control program, ethics committee approvals will be required. Because of the rapidly developing science in this field, it is not possible to predict precisely what tests will be performed with these samples. In addition, many important discoveries are likely to be made through the analysis of human DNA and RNA and thus informed consent for this use will also be sought, which will include subsequent testing for genetic markers.

7.1. CENTRAL SPECIMEN REPOSITORY

The National Institute for Research in Tuberculosis (NIRT) in Chennai will be the Central Repository for the India RePORT sites acting under contract to the DBT. NIRT will provide centralized training and laboratory support to the CRUs as needed.

Specimens for Long Term Storage at the Central Repository

All processing and aliquoting of samples will take place in the CRU laboratory prior to sending samples to the Central Repository (Table 4). Samples will then be shipped to the Central Repository where they will be curated, managed, and stored for a maximum of 10 years. Samples will only be destroyed with written permission of the sponsor and according to local regulatory guidelines.

The sites will be provided with sample collection kits. Details for the collection, processing, storage, and shipping of samples collected as part of the Common Protocol are presented in the RePORT-India Consortium Manual of Operation (MOP).

TABLE 4: BIOSTORAGE STUDY SPECIMEN COLLECTION AND STORAGE CHART (ADULTS AND CHILDREN)

Specimens Type		Adults and Children (years)	Children (< 5 years)[a]
Whole blood (PAXgene tubes)		2.5 mL	2.5 mL[b]
Whole blood (QuantiFERON-Gold InTube)		3.0 mL (1.0 mL/tube)	3.0 mL (1.0 mL/tube)
Whole blood (Genotyping)		10 mL	**DO NOT COLLECT IF VOLUME EXCEEDS REGULATORY LIMITS**
PBMCs		8.0 mL (BD CPT™)	4.0 mL (BD CPT™)
Plasma		Harvested from BD CPT™ (PBMC) tubes above	Harvested from BD CPT™ (PBMC) tubes above
Urine		Spot urine (10 mL)	Spot urine (10 mL)
Sputum		Whatever volume is possible to collect	Whatever volume is possible to collect
Extracted host RNA		Prepared from PAXgene tube	Prepared from PAXgene tube
M.Tb isolate	**Cohort A**	Subculture of original Mtb isolate and relapse or failure isolate.	Subculture of original Mtb isolate and relapse or failure isolate.
	Cohort B	Subculture of confirmatory Mtb isolate from each subject who develops active TB	Subculture of confirmatory Mtb isolate from each subject who develops active TB

[a] Refer to weight chart for maximum blood volume collection limits in MOP. Indicated blood collection volumes are intended for children ≥ 5 kg (or 11 lbs) .

[b] Exclude from collection if combined study and biobank blood volume limit is exceeded –Maximum combined blood volume collection is limited 2.5 mL/kg.

8. ASSESSMENT OF OUTCOME

Aim 1:To examine the host and microbial factors associated with TB treatment outcomes

- Primary outcome for Aim 1 will be a composite poor treatment outcome, defined as treatment failure, recurrence or death within 24 months of TB treatment initiation.
- Secondary outcomes will include time-to-sputum AFB negativity, time-to-culture negativity, and time-to-detection of MTB using liquid sputum culture on mycobacteria growth indicator tubes (MGIT).

Aim 2: To investigate the host and microbial factors associated with the progression from infection to active TB disease

- Primary outcome is incident TB within 24 months (best outcome to assess what factors are associated with the risk of progressing to active TB)
- Secondary outcome will be proportion with co-prevalent TB at time of initial household screening.

Aim 3: To explore the host and microbial factors associated with MTB transmission

- Primary outcome will be incident infection defined as conversion of TST or QGIT or at entry a positive TST (> 15mm) or QGIT in a child <5 years.
- Secondary outcome will be prevalent active TB at entry or incident TB among HH contacts that have the same DNA fingerprint using MIRU based genotyping at NIRT.

8.1. OUTCOME MEASURES FOR ACTIVE TB COHORT (COHORT A)

Participants' samples from Cohort A will be saved in the bio-repository as they are likely to yield valuable information regarding biomarkers that correlate with changes over time with treatment, presence of clinical markers of disease severity (e.g., cavitary lesions on chest radiograph), and final outcome. Participants with relapsed or failed TB will be of special interest, reflecting a poor treatment outcome. All participants will be categorized by disease outcome status and by treatment completion status.

Bacteriologic outcomes, drug-susceptible (DS) TB:

1. **DS bacteriologic cure:** By the end of the RNTCP-specified standard, first-line multi-drug TB therapy, the participant has two or more consecutive respiratory secretion cultures negative for M. tuberculosis, and no evidence of bacteriologic failure.

2. **DS bacteriologic status indeterminate**: By the end of RNTCP-specified standard, first-line multi-drug TB therapy, the participant has resolution of symptoms of tuberculosis, but has not had two or more consecutive respiratory secretion cultures performed to establish cure status.

3. **Bacteriologic failure:** By the end of RNTCP-specified standard, first-line multi-drug TB therapy, the participant has one or more specimens from the respiratory tract or extra-pulmonary site that are culture-positive for M. tuberculosis in either liquid or solid media (with any colony count), when the culture has not been determined to be a false-positive culture.

   DS bacteriologic recurrence Among participants who did not experience a clinical (as described below) or bacteriologic failure of therapy, the participant has a clinical specimen collected from any site during the follow-up phase that is culture-positive for M. tuberculosis in either liquid or solid media (with any colony count), when the culture has not been determined to be a false-positive culture.

Clinical outcomes: Participants who do not meet bacteriologic criteria to establish their final status may be assigned a clinical outcome.

1. **Clinical response:** A participant who has resolution, by the end of therapy, of symptoms attributable to tuberculosis.

2. **Clinical failure:** The persistence, progression, or recurrence of signs and symptoms of tuberculosis (fever, sweats, productive cough, documented weight loss, worsening of chest radiograph, progressive evidence of extra pulmonary tuberculosis) that is determined by the principal investigator (PI) to be due to tuberculosis and not due to another underlying cause.

3. **Clinical relapse:** Clinical or radiographic evidence of active tuberculosis after completion of therapy but before the end of the follow-up phase.

In addition to disease outcome status, participants will be categorized regarding completion of treatment status, defined as follows:

Completion of therapy status:

1. **Completion of adequate therapy:** Considered accomplished when the patient is not a treatment failure and has received the number of doses of RNTCP-specified multi-drug anti-TB therapy recommended, depending on their initial disease status categorization (DS or DR).

2. **Incomplete**: Treatment is considered incomplete if the subject has defaulted from treatment, defined as treatment interruption for two or more consecutive months.

8.2. OUTCOME MEASURES FOR HH CONTACT COHORT (COHORT B)

Active TB is the primary outcome measure of interest in this cohort and will be defined as the following:

a. No TB: Participant had no indication of MTB (pulmonary or extra-pulmonary) over follow up period

b. Definite case: Culture-confirmed TB from any anatomic site.

c. Probable case: Adult (≥ age 14) with symptoms of TB plus acid-fast bacilli seen on microscopic examination of sputum or biopsy specimen, but without culture-confirmation.

d. Probable case: Child (<14)defined as:

   i. Symptoms consistent with TB, specifically fever or cough for 2 weeks, or loss of weight or failure to gain weight normally, plus a history of contact with a suspected or confirmed case of active TB AND

   ii. Acid-fast bacilli seen on microscopic examination of sputum or biopsy specimen

   OR

   Culture-negative after 2 attempts at sputum collection, using sputum induction, nasopharyngeal aspirates or gastric aspirates and have chest radiography that is consistent with intra-thoracic disease due to Mycobacterium tuberculosis.

   iii. AND there is at least 1 of the following:

      1. A positive clinical response to standard multidrug anti-tuberculosis treatment OR

      2. Documented exposure to a case of active TB.

   iv. AND immunological evidence of M. tuberculosis infection (e.g, reactive TST or positive IGRA)

e. Possible case: Adult or child diagnosed with extra-pulmonary TB based on radiographic or other evidence, but without culture-confirmation.

Incident MTB infection is the secondary outcome measure in this cohort and will be defined as a positive TST or QGIT in children < 5 years or seroconversion of TST or QGIT among HH contacts during follow up.

8.3. COHORT B TO COHORT A TRANSITIONS

If at any time during the study period a Cohort B participant develops active TB disease, he/she would be approached for consenting to be part of Cohort A. After the consent process, if he/she were to agree then a new SIN would be assigned with the Cohort A identifier. All Cohort B visits would cease and the participant would follow the schedule of evaluations for Cohort A, with the exception of the data that has already been collected for the household. The eMOCHA system would auto-fill information that does not need to be repeated in order to minimize the burden on the participant.

9. STATISTICAL CONSIDERATIONS

9.1. SAMPLE SIZE AND ANALYSIS PLAN

Aim 1. The Active TB Cohort will include 800 adult PTB cases, 200 adult EPTB cases and 200 pediatric TB cases. The primary analysis set will be 800 adult PTB patients. Secondary analysis sets will include EPTB and pediatric TB.

Primary outcome is poor treatment outcome defined as failure, recurrence or death. Secondary outcomes will include time to AFB smear negativity, time to sputum culture negativity; time to detection of MGIT positivity. Host factors age, sex, DM, HIV, IAP exposure, tobacco exposure, malnutrition, and mental health will be key predictors for poor treatment outcomes.

Person-years of follow-up will be calculated for each member of the analysis set. Of TB patients who experience the outcome of interest, person-years of follow-up will be calculated as number of years between date of enrollment and date of event. Among those without the outcome person-years will be the number of years between enrollment and last date of follow-up. Crude treatment failure rates and 95% exact Poisson confidence intervals in the presence and absence of primary risk factors will be calculated. Adjusted treatment failure rate ratios will be estimated using multivariate Poisson regression models with person-years of follow-up as an offset. The Poisson regression models will also investigate interaction between individual and HH factors that might influence treatment outcomes.

A secondary subgroup analysis (may not be sufficiently powered) by enrollment sites, NIRT and BJMC, will be performed by adjusting the level of significance using a Bonferroni adjustment.

Aim1 Power. For Aim 1, we expect 20% (n=160) of 800 adult PTB cases will have the composite primary outcome (2% will fail, 6% will die, and 12% will have recurrence). This study will have at least 90% power at 5% level of significance to identify host and microbial risk factors (enumerated in section 3B3), with a prevalence of 10% - 40%, associated with poor treatment outcomes with relative risk ratio ranging from 1.5 to 6.0 over 24 months of follow-up.

Aim 2. The analysis set is individuals in the HH Contact Cohort: 3200 HH contacts of 800 PTB patients.

The primary outcome is diagnosis of active TB among individual HH contacts within 24 months of follow-up. Index PTB cases from the Active TB Cohort will form clusters and individuals within the HH of the index case are correlated. The association between host and microbial factors with progression from infection to active TB disease will be estimated using random effects.

Poisson regression models with index PTB cases as random effects. Random effects models will address the correlation of individuals within the HH. Relative risk ratios and 95% confidence intervals for host and microbial factors will be estimated from the models and will have a cluster-specific interpretation.

Aim 2 Power. We expect approximately 3200 HH contacts will be followed for an average duration of 2 years. We anticipate 85% (n=2720) of them will be HH contacts of a smear positive PTB case, 15% (n=480) will be contacts of a smear negative PTB. We estimate that at least 40% (n=1280) will be latently infected.1 We estimate that 320 (25%) of them would be recently infected and have a 15% 2 year risk of developing active TB.108,134 This would result in 48 active TB cases. We expect a background rate of active TB (regardless of latent TB infection) 0.37% per year, yielding an additional 24 cases. This yields an estimated 72 incident active TB cases.

Aim 3. The analysis set will be HH members of index PTB cases.

Primary outcome will be incident infection will be defined as positive TST or QGIT in children < 5 years or seroconversion of TST or QGIT among HH contacts. Secondary outcome will be prevalent or active TB with same MTB MIRU genotype.

Within household transmission rates will be calculated. Risk factor and covariate information collected on HH contacts will be used in the analysis. In addition the analysis will be adjusted for independent variables measured at HH level. A multivariate random effects Poisson regression model will be used to estimate the adjusted relative risk of MTB transmission to HH contacts in presence of host and microbial factors. The model will be adjusted for enrollment site (NIRT & BJMC).

10. SUBSTUDIES

10.1. PK SUB STUDY

1a) To evaluate the population PK of H, R and Z among Indian patients with TB, taking into account actors known to affect H,R, and Z concentrations (NAT2 or SLCO1B1 genotype, BMI, HIV status, etc.)and examine the relationship between H, R, Z concentrations and poor treatment outcome (failure, relapse, acquired resistance).

We hypothesize sub-therapeutic concentrations of R will be common among patients with TB in India, but given the high prevalence of the slow H metabolizer genotype among Indian patients, H concentrations will be in the target range for most patients. Poor TB treatment outcome (failure, relapse, or acquired resistance) will be associated with low concentrations of H, R, Z among patients with PTB in India. From newly-diagnosed PTB patients in Active TB Cohort, serial sputum samples will be collected and archived as per SOE Table 5. A 2-hour post-dose plasma sample for HRZ analyses will be collected at months 1,, and 5and stored. Patients will be followed at least 12 months after completion of TB treatment for outcomes of interest.

PK samples from both sites will be analyzed at NIRT. Population PK/PD models will include cofactors that are known to influence drug concentrations such as genetic polymorphisms (NAT2 genotype or others), BMI, age, sex, and HIV to evaluate the relationship between H, R or Z concentrations and treatment response. In addition, at NIRT, minimum inhibitory concentration (MIC)

determinations over time will be performed on the stored samples of those patients with suboptimal treatment outcomes to see the evolution of drug resistance over time.

1b) To explore a novel assay to measure PK of first line TB drugs from small hair samples and correlate these concentrations with self-reported levels of TB drug adherence, plasma drug concentrations and treatment outcomes.

We hypothesize that H and Z concentrations in hair will correlate with self-reported adherence to TB drugs, with plasma concentrations of these drugs, and with treatment outcomes among adults receiving first-line treatment for PTB. We will collect small hair samples from Active TB Cohort patients for an innovative additional measure of therapeutic drug monitoring of TB drugs. In addition to ease of sample collection and storage, hair concentrations of drug reflect uptake from the systemic circulation over weeks to months and provide an advantage over single plasma monitoring in reflecting long term drug exposure [56], which has also been predictive of treatment outcomes [57-59]. Dr. Monica Gandhi and her team at UCSF have recently developed novel hair assays to analyze H, Z concentrations in small hair samples to serve as additional PK measures in our study. Dr. Gandhi will initially assess the hair H,Z concentrations at UCSF and work with NIRT to build local capacity to perform the assay in India.

10.2. FUNDAMENTAL/TRANSLATIONAL SCIENCE LABORATORY STUDIES

Using the samples collected from C-TRIUMPh cohorts, investigators at IGIB, NIRT, CRF, THSTI,JHU, and UW propose the following studies if approved by C-TRIUMPh Leadership and Scientific Working Groups. Funding for these studies will leverage existing Indo-US funds at these institutions and from new investigator initiated grants.

a) Whole blood transcriptome for genomic studies- Drs. Gokhale, Singh and Rao at IGIB
b) Host proteomic biosignatures in serial serum samples from TB cases- Drs. Gokhale, Singh, and Rao at IGIB
c) MTB Culture isolates. Dr. Singh at IGIB.
d) Whole blood and EPTB samples transcriptome for clinical presentation of TB disease- Drs. Gokhale (IGIB) and Jain (JHU).
e) Biomarker discovery with novel antigens for diagnosis of latent and active tuberculosis – Dr. Raja at NIRT
f) Determination of host genetic variants and clinical outcomes in TB patients-Drs. Ramakrishnan and Szumowski at UW.
g) Quantification of novel host proteins from PBMCs from TB cases, HH contacts with new MTB infections, and controls-Drs. Lamichane (JHU) and Rao (IGIB)
h) Identify signatures of latency and active tuberculosis in Indian population- Dr. Agarwal (THSTI).
i) MTB-specific immunity using stored PBMCs and QGIT supernatant- Dr. Hanna at NIRT

j) Host inflammation, oxidative stress, IAP, nicotine and TB outcomes using stored blood, sputum, urine and hair-Drs. Salvi (CRF) and Biswal (JHU)

11. CLINICAL MONITORING STRUCTURE

11.1. CLINICAL SITE MONITORING

Internal QA procedures will be put into place to ensure the quality of study related activities. If a participant signs a consent form, QC of the consent form will be done before the participant leaves allowing immediate correction of any errors. Informed consent checklist would be utilized for the same. Study Clinicians along with Research Nurses will be responsible for the quality control activities in the clinic. CRFs will be completed by Study Clinicians and counselors and checked for completeness and accuracy by Study Nurses or other trained staff. Study Nurse/staff will use a visit checklist, and referring to CRFs will also ensure all tests and procedures have been completed before the participant leaves the clinics or the outreach team leaves the house. The Laboratory & Specimen Working Group will be responsible for overseeing the QC procedures of the CSS labs with support from the site Lab Research Officers. Number of Consent violations, percentage of data completeness of CRFs and other data forms, retention rates and reasons for missed visits will be monitored with the help of the Data Management Group.

11.2. ACCRUAL AND RETENTION MONITORING

Study sites will develop a screening log based upon the study inclusion/exclusion criteria. Study coordinators and specified OWGs are responsible for reviewing the accrual reports on an ongoing basis during the study accrual period and taking action as necessary to ensure that accrual targets are met. CSS oversight personnel are responsible for daily monitoring of enrollment and referrals and will conduct weekly meetings to discuss and implement approaches to ensure high enrollment rates. Outreach team will arrange periodic meetings with cab to discuss recruitment strategy. If at any time these reviews identify areas for improvement, OWGs will follow-up with relevant study staff to ensure that any oversight or deficiency is appropriately addressed. All actions taken will be documented throughout the study period; participants will be continuously instructed by counselors and study clinicians on important issues including: study objectives, follow-up visits, adherence to medication, healthcare education, transmission risk reduction, contact information. After each visit is completed, the study nurse/HH outreach team will schedule the next visit in the participant's study card. The counselor will communicate visit reminders to study participants prior to study visits. Participant's contact details will be recorded in the participant's locator form and kept in the participant's file. The counselor will verify the participant contact details at every visit. if participant doesn't show up on their scheduled visit within the window period as per protocol after the scheduled visit, the counselor will contact the participant to find out the reason and reschedule the visit as soon as possible.

12. DATA HANDLING AND RECORD KEEPING

12.1. DATA MANAGEMENT AND MONITORING OF DATA QUALITY

Dr. C. Ponnuraja & Mr. T. Kannan NIRT along with Dr. Gupte, BJMC will draft a data quality management plan prior to study accrual. All enrolled participants in the three study cohorts will be identified by a unique study identification number (SIN). The SIN will be formatted to identify the study site, study cohort, and link the index PTB cases to their HH contacts. The SIN of cohort 2 participants will be formatted to identify its index case. The site data manager will maintain a log in MS Excel with participant names linked to the SIN. SIN will be preprinted on labels to be pasted on study CRFs.

12.2. DATA CAPTURE METHODS

CRFs will be standardized, structured, and will be pretested before implementation. Source documents will be designed specifically for the study and data will be collected on the source documents at the study sites. Study personnel responsible for completing the source documents will ensure that all fields are completed before the participant leaves the study site. eMOCHA will be used to collect the study data starting early 2015 The **e**lectronic **Mo**bile **C**omprehensive **H**ealth **A**pplication (eMOCHA®) is an application, developed by the Johns Hopkins Center for Clinical Global Health Education. eMOCHA® is a secure, highly flexible and adaptable, mHealth application developed by the Johns Hopkins Center for Clinical Global Health Education. While the system is being finalized, paper CRFs will be used for data collection. CRFs will be completed by trained clinicians, nurses or counselors using the source documents. The CRF fillers will ensure that all fields are completed. A 10% QC check will be conducted by the site data manager daily to cross-check the data on the CRFs and source documents for accuracy. The study coordinator will conduct a monthly QA of the CRFs and source documents and findings will be documented. The data will be entered in the computer by the Data Entry Operators. The data manager will work with the study coordinator at each CSS to identify additional training requirements depending upon the QA/QC findings.

12.3. DATABASE

Since the study is conducted at 2 CSS, a web-based password protected data management system will be used. Data manager at both sites will have administrative rights on managing the database. Data entry will be programmed to catch missed skip patterns and logical data sequence at the time of data entry. The delinquency program to identify expected CRF's that are missing from the database will be run by the site data managers on the daily basis. After double data entry an error check program will identify data entry errors. Logical data check program will be executed by the data manager within 48 hrs of data entry and the respective sites will have 1 week to resolve errors in the data checks. Logical queries will be run by the site data manger once every 2 weeks and the site will have 3 weeks to resolve these queries.

13. PROTECTION OF HUMAN SUBJECTS

Rationale of the study: The purpose of this study is to evaluate the risk factors for TB among adults and children in India. This study will also look into new ways to diagnose and treat TB disease. It will also assess household contacts for presence of active TB and for risk for TB transmission to them from the active index case. In addition, the study will also compare the features among people who are exposed to TB with those who are not exposed to active TB patients, thus evaluating who is at risk for the development of the active TB and who is not.

Study Subjects: The study will enroll individuals with active TB and those with either no active TB or who are not actively seeking care for active TB. Although the second group are not actively seeking medical care, they will be undergoing some additional tests beyond the usual clinical practice, however these tests will provide information about individual's risk to develop TB and therefore may provide an opportunity to that individual to get early medical care.

Role of Study sites**:** The study has been planned as multi-centric, and will be done at 2 sites: BJMC CTU in Pune and NIRT in Chennai. The NIRT clinical site will be enrolling cohort patients attending RNTCP clinics in 2 TUs of Tiruvallur district namely, Velliyur and Poonamalle TUs.

Potential Risks: Taking blood may cause some discomfort, bleeding, or bruising where the needle enters the body, lightheadedness, and in rare cases, fainting or infection. Blood volume collected will be age appropriate and will follow international guidelines. HIV testing (when done) will cause physical discomfort, but our trained phlebotomists will take utmost care to limit this risk. The study participants may feel embarrassed at the sensitive nature of some of the questions especially with regard to alcohol and smoking.

Adequacy of protection against risks: The study site will make every effort to protect privacy and confidentiality of participants. Trained counselors and phlebotomists will take utmost care to limit the risk arising from sociological issues and blood draws. The study subjects will be able to withdraw from the study at any time. Lack of participation in the study will not have any adverse effect on their future medical care. The study investigators will answer any questions regarding the study at any point of time. The study subject or the parent/guardian of the child in the study will be provided the contact details of the principal investigator, or his representative, for clarification of any details.

Recruitment and informed consent**:** Study consent forms will be provided to eligible active TB cases, their household members and control neighborhood household members by study staff in the local language. For illiterate participants, study staff or a counselor will read the consent in the local language, in the presence of an impartial witness. Participants will be informed of the objectives of the study, the procedures involved and the risks and benefits. Their option to not enroll, as well as the possibility of

withdrawing from the study, will be made clear to the participants. They will be given sufficient time to think about their willingness to participate in the study. The participants will also be given the opportunity to ask questions. Informed written consent will be obtained from the subjects, parent/guardian children before commencement of the study. In addition, assent will be obtained from children aged 8 years and above. The study will commence after obtaining clearance from the Institutional Ethics committees of NIRT.

Confidentiality: All patient data forms, including consent forms will be kept in a locked filing cabinet accessible only to the project personnel. The data forms will not be shared with anyone other than the research team. The database will be password protected of patient-specific data. However, all personal identifiers will be deleted from the final research database. JHU and other members of the research team, who are not involved in direct project work, will not have access to any patient personal health identifiers. Electronic research data will be kept in a password-protected computer with access provided only to the study personnel.

Benefits to Subjects: The proposed study may benefit individual active TB cases as the study will perform testing beyond the usual clinical practice such as detecting resistant TB. The study will also screen family members of the Active TB Cohort participants for TB and risk factors for TB. In addition, the control cohort will get additional information on their health, including risk factors for active TB. In addition to the benefits described above, all individuals will have access to tests that detect TB and this may result in getting early access to care.

Provisions in Case of Accident/Injury: If the participant is injured as a result of being in this study, he/she will be given immediate treatment for their injuries. He/she may not have to pay for care and treatment that is available in this hospital; however he/she may have to pay for care and treatment if he/she is referred to another hospital by the study doctor. There is no program for compensation. He/she will not be giving up any legal rights by signing the consent form.

Importance of knowledge to be gained: Information learned from this study may help to diagnose and treat TB in the community and may improve the ability of health care workers to diagnose and treat TB. Understanding the relative importance of modifiable risk factors in the development of TB will help to inform which should be prioritized for public health interventions to reduce the burden of TB in India and other settings. We will be able to provide much needed data on interaction and combined risk of these factors which can be used for improved TB epidemic modeling.

Inclusion of women and children: Because the study will take place among residents of India, all study subjects will be of Asian Indian race. No person will be excluded due to ethnic, religious, socioeconomic or racial characteristics. Prisoners will be excluded from the study as well as those who are deemed incapable of providing informed consent and those who have a serious illness or condition in which the opinion of the site investigators deem would prevent adequate follow-up. Following is the plan for enrollment for children and women into the three cohorts.

14. C-TRIUMPH FUTURE PROJECTS AND PUBLICATION POLICY

C-TRIUMPh Leadership Group (LG), consisting of Dr. Soumya Swaminathan, NIRT and Dr. Amita Gupta, JHU, will oversee the tracking of the scientific working group (SWGs) These are required for proposed analyses using existing datasets, collection of new data, and/or use or collection of specimens. These proposals will be discussed on the SWG calls, reviewed by the data management and laboratory management WGs to evaluate the science and the feasibility of the proposed study. These will then be submitted electronically using a standard submission form and reviewed in a timely fashion by the designated SWGs and then by the LG. The time from submission to project initiation and completion will be tracked by SWG chairs and LG, and strategies to ensure publication delays are minimized will be developed and employed.

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